



TITLE:

**SIMPLE TECHNOLOGY of STARCH
HYDROLYSIS by USING
PULLULANASE ENZYME**

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OBJECTIVES



To investigate the effect of:

- Temperature on starch hydrolysis.
- pH on starch hydrolysis.



PROBLEM STATEMENT

- The use of pullanase as the enzyme in tapioca and sago in starch hydrolysis has not been widely explored.
- Existing method/current technology of starch hydrolysis is energy and time-consuming *(Costanzo Bertoldo, Fiona Duffner, Per L. Jorgensen, and Garabed Antranikian, 1999)*

PROBLEM STATEMENT



- Syrup produce by α -amylase; have combination of glucose, maltose & other sugar, gave the low yield of glucose *(G.S Ayernor, T.K Hammond, A. Graffham, June 2002)*

- The neopullulanase enzyme from *Bacillus stearothermophilus* hydrolyzes α -(1-4)-glucosidic linkages of pullulan to produce panose *(Takashi Kuriki, Michiyo Yanase, Hiroki Takata, Yoshiaki Takesada, Tadayuki Imanaka, and Shigeta Okada, 1993)*

PROBLEM STATEMENT

- Starch liquefied with amylase and saccharified by glucoamylase gave low yield of production of glucose

(*Yoshiyuki Takasaki, 1994*)

SCOPE



- Two types of sources of starches ; tapioca and sago will be used.
- Pullanase from *Bacillus acidopullulyticus* will be utilized as enzyme
(C.W.Wong,S.K.S Muhammad,M.H Dzulki-fly,N. Saari,H.M Ghazali,October 2005)
- The enzymatic starch hydrolysis will be conducted in 2L Biostat fermenter
- The different effect of pH will be investigated(5.0, 5.5,6.0)
- The different effect of temperature will be investigated
(50°C,55°C,60°C)

METHODOLOGY



Mixed 10% (w/v) dry solid of tapioca starch / sago starch and acetate buffer
(G.S Ayernor, T.K Hammond, and A.Graffham, June 2002.)

Adjusted the mixture by using Hydrochloric Acid/ Sodium Hydroxide to the desired pH

Pullanase were added to **LIQUEFICATION & SACCHARIFICATION** the starch.

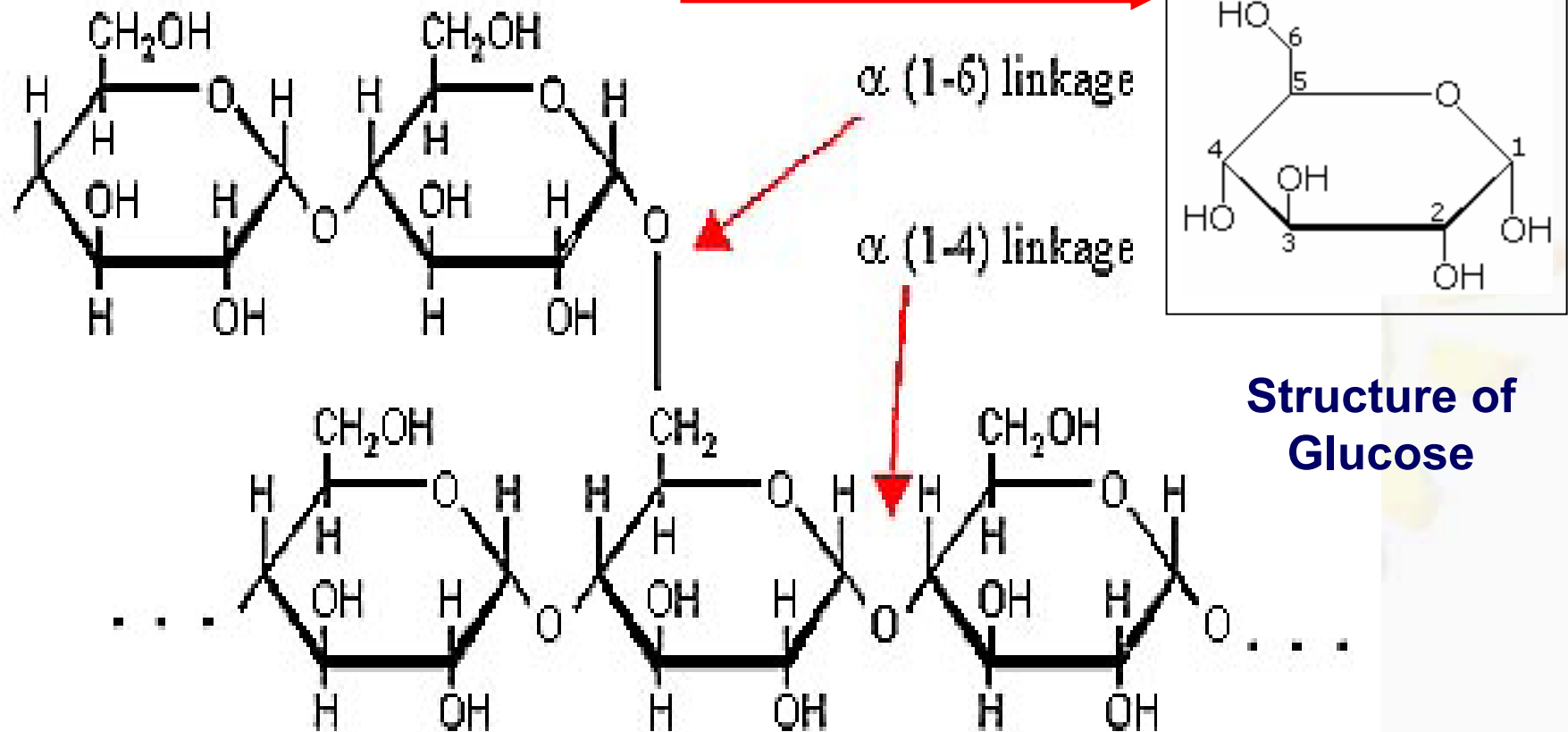
- 1-2 hours
- Impeller speed 300rpm
- 0.2% enzyme (*Chiu, Chung Wai,1990*)
- temperature (50, 55,60)

The samples were collected every 25 minutes and lastly determined the concentration of the glucose in the sample.

The method performed as reported by ROSNANI SAIDAN in her report – Simple Technology of Starch Hydrolysis by Using α -Amylase and Amyloglucosidase enzymes simultaneously
May 18, 2007



Structure of Starch



METHODOLOGY

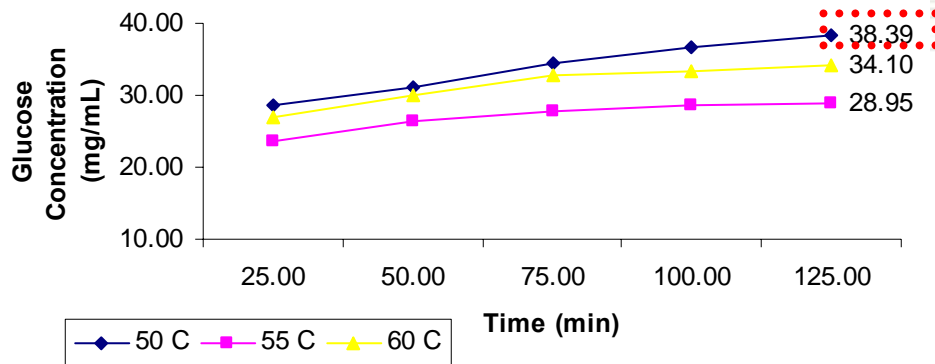


DETERMINATION OF GLUCOSE CONCENTRATION

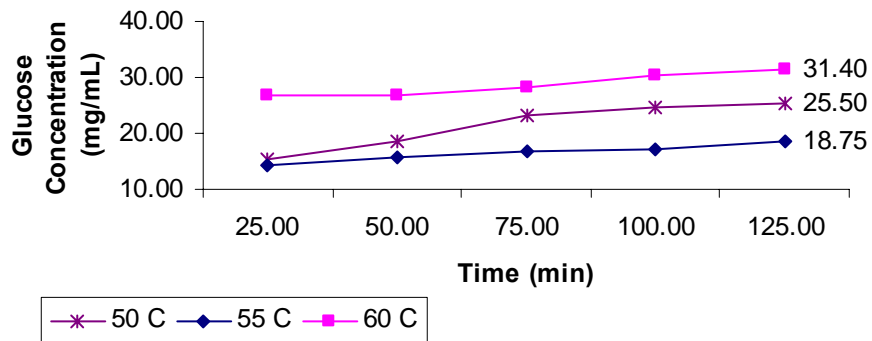
1. Dinitrosalicylic acid (DNS) Method
2. High Pressure Liquid Chromatography (HPLC)

FINDINGS

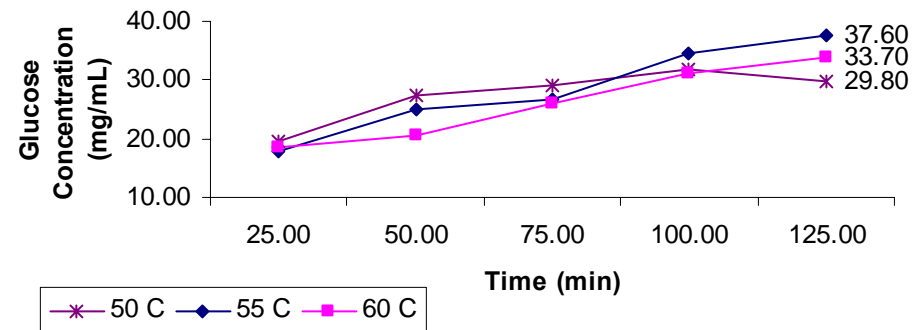
Effect of Temperature of Sago Starch Hydrolysis for pH 5.0



Effect of Temperature on Sago Starch Hydrolysis for pH 5.5



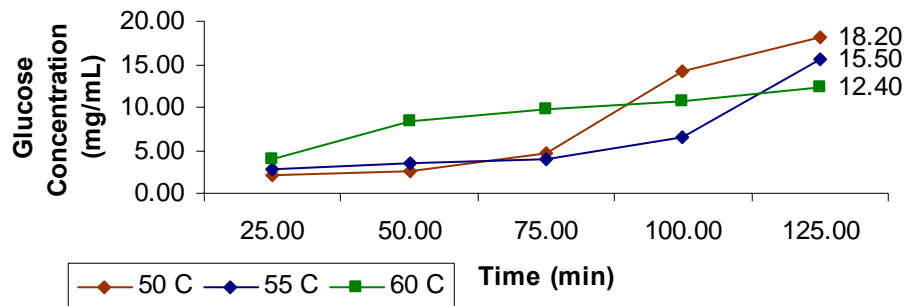
Effect of Temperature on Sago Starch Hydrolysis for pH 6.0



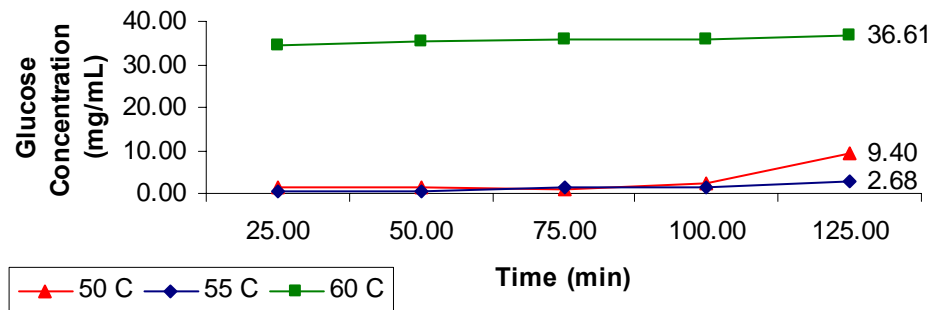
FINDINGS



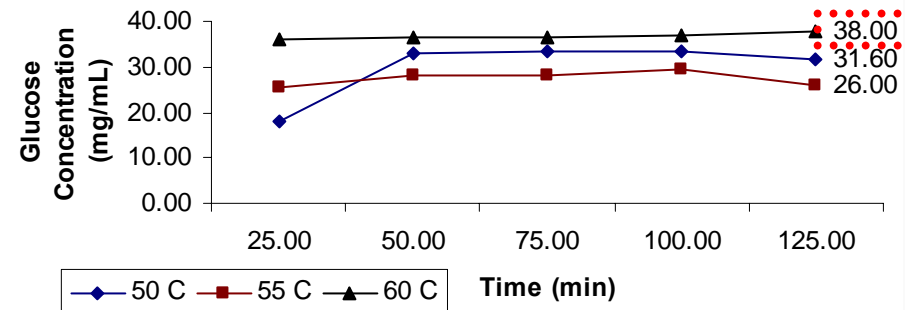
**Effect of Temperature on Tapioca Starch Hydrolysis
for pH 5.0**



**Effect of Temperature on Tapioca Starch Hydrolysis
for pH 5.5**



**Effect of Temperature on Tapioca Starch Hydrolysis
for pH 6.0**



FINDINGS



pH	Temperature		
	50	55	60
5.0	28.75	23.75	31.07
	31.07	26.43	31.43
	34.46	27.68	32.68
	36.61	28.75	33.21
	38.39	28.95	33.57
5.5	15.36	15.71	26.61
	18.75	15.89	26.79
	23.21	16.61	28.04
	28.04	17.32	30.36
	35.71	18.75	32.32
6.0	15.36	17.68	18.39
	28.93	26.43	20.71
	31.61	26.61	25.89
	31.96	34.64	33.21
	32.86	37.86	35.54

Table 1

pH	Temperature		
	50	55	60
5.0	0.18	2.86	3.93
	0.54	3.39	8.39
	2.32	3.93	9.82
	14.11	6.61	10.71
	17.50	15.71	14.46
5.5	0.18	0.36	35.18
	0.71	1.07	35.89
	1.07	1.25	36.07
	1.25	1.61	36.43
	8.21	2.68	36.61
6.0	18.04	28.75	35.18
	33.04	30.00	36.07
	33.93	30.71	36.61
	34.29	31.25	36.96
	35.89	32.50	38.00

Table 2

Table 1: Yield (% g glucose/g starch) obtained during the Sago starch hydrolysis for different pH and Temperature

Table 2: Yield (% g glucose/g starch) obtained during the Tapioca starch hydrolysis for different pH and Temperature



FINDINGS:

Sago Starch :

Highest concentration of glucose obtained from starch solution with pH 5.0 at 50°C = 38.39 mg/mL

Yield = 38.4 % (g glucose/ g starch)

Tapioca Starch :

Highest concentration of glucose obtained from starch solution with pH 6.0 at 60°C = 38.00 mg/mL

Yield = 38.0 % (g glucose/g starch)

DISCUSSION



LOW YIELD??? WHY???

- Unsuitable experiment condition for the starches used. – *mostly use corn starch and potato starch*
- Low concentration of enzyme (*C.W.Wong,S.K.S Muhammad,M.H Dzulkifly,N. Saari,H.M Ghazali,October,2005*)

RECOMMENDATION



- Amylopullulanase can be utilized as enzyme instead of pullulanase – can hydrolyzed both glucosidic bond
 - *(Yuji Hatada, Kazuaki Igarashi, Katsuya Ozaki, Katsutoshi Ara, Jun Hitomi, Tohru Kobayashi, Shuji Kawai, Tomoyoshi Watabe, and Susumu Ito, 1996)*
- Higher enzyme concentration -5% (v/w) enzyme
 - *(C.W.Wong, S.K.S Muhammad, M.H Dzulkifly, N. Saari, H.M Ghazali, October, 2005)*
- Lower starch solution concentration – < 10%: >5 % (w/v) considered very high viscous .
 - *(C.W.Wong, S.K.S Muhammad, M.H Dzulkifly, N. Saari, H.M Ghazali, October, 2005)*

CONCLUSION



- Concentration of glucose obtained:
Tapioca Starch- **38.00 mg/mL**
Sago Starch - **38.39 mg/mL**
- Optimum pH and temperature:
Tapioca Starch- **pH 6.0 at 60°C**
Sago Starch - **pH 5.0 at 50°C**
- Yield obtained:
Tapioca Starch – **38.0%**
Sago Starch – **38.4%**



THANK YOU

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